Chapter XI

The Reproduction of Viruses: A Comparative Survey

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I. Virus Infection as Infective Heredity

A. Virus Multiplication, Cell Multiplication, and Cell Growth

The problem of virus growth has features that differ from those of growth problems in cells and in multicellular organisms. Multicellular organisms grow by fission of cells and multiply by releasing some more or less specialized cells, which give rise to new individuals. Multiplication of cells is itself, in turn, the culmination of intracellular processes, during which specific subcellular structures and molecular species increase in number. The replication of these subcellular elements represents cell growth, ultimately leading

to cell multiplication. The thesis of this chapter will be that virus multiplication as a biological process belongs on the level of the replication of subcellular elements, that is, on the level of cell growth rather than of cell multiplication.

In the same way as the morphogenesis of multicellular organisms must be interpreted in terms of the creation of organized patterns of specifically differentiated cells, so must the growth of cells be interpreted in terms of the formation and maintenance of organized patterns of specific molecules and macromolecular complexes. The morphogenesis of these patterns and the synthesis of their constituent parts are the subject matter of cytochemistry. The study of virus multiplication is a branch of cytochemistry; a remarkable branch, in fact, since it presents unique opportunities for the study of some cellular constituents in isolation in a fully native, functional, undegraded form, and of their transition from the isolated, inert state to the integrated, functional state as parts of the living protoplasm.

That virus multiplication is not a process homologous to cell multiplication is suggested immediately by the structure and composition of virus particles. All cells capable of multiplication, no matter how different their origin, size, and structure, contain certain essential chemical constituents—including proteins and nucleic acids both of the ribose (RNA) and deoxyribose (DNA) types—and certain essential organelles—nucleus, mitochondria, microsomes, cell membranes. Instead, as discussed in Chapters 3 and 6, most virus particles lack one or more of the basic chemical constituents of cells. Their composition and organization are much simpler than those of any cell.

There is great variation in these respects among different groups of viruses. Viruses are grouped together taxonomically on methodological rather than biological criteria. There is no reason to assume that they represent a naturally related group. The ability to invade living cells from outside and to multiply within them, which is a major criterion used to define viruses, may well be common to a variety of unrelated elements. The size of virus particles, another criterion used to group viruses together, ranges over a factor of 10,000 in mass and is no indication of natural relationship.

Whatever basic similarities exist among all viruses or among groups of them can be revealed only by the methods of cytochemistry, that is, by the study of the structure and composition of the virus particles and of the chemical and physiological events that their presence and multiplication produce in the cells. The relevant facts are discussed in detail in other chapters of this book. Here we are concerned only with tracing basic similarities and outstanding differences among viruses as revealed in their processes of multiplication and with deciding whether any generalizations appear justified by our present knowledge of these processes.

Four main approaches provide information on virus multiplication: (1) the *kinetic* approach, which follows the increase in numbers of virus particles by measurements of infectivity or of other specific virus properties; (2) the *cytochemical* approach, which studies the structural changes in cell organization accompanying virus production and the localization of viral materials within infected cells; (3) the *biochemical* approach, which analyzes the biosynthesis of virus constituents, their origin, fate, and continuity, and the alterations in cellular functions correlated with virus multiplication; and (4) the *genetic* approach, which traces the continuity and variation of the specific determinants of virus properties, their organization within the virus, and the interactions between viral and cellular determinants of specificity.

All these approaches must be utilized, and their results correlated, in order to obtain a complete picture of virus multiplication. Only for some bacteriophages has such a program of research been carried out to any great extent; a number of other viruses are now being studied in similar ways.

B. Virus as Genetic Determinant

The results of these studies have led to what we consider as two central generalizations: the concept of virus multiplication as an altered pattern of biosyntheses in an otherwise functional cell; and the concept of the virus as contributing to the cell a set of genetic elements, which initiate and determine the new biosynthetic pattern.

We shall first elaborate these concepts; then, outline the evidence available from various areas of virology to support and specify them; and finally, discuss briefly the meaning of these concepts for the interpretation of cytomorphogenetic and pathogenic effects of viruses and of the relationship between viruses and cellular constituents.

C. Virus Replication and Virus Maturation

Virus action within the host cell consists essentially in the production of abnormal or unusual cellular products as a result of exact specifications contributed by the virus itself. The unusual cell products may include virus particles, virus-related materials, and also cell constituents that have no obvious similarity to the component parts of the virus particles as observed in the free state. Virus infection can properly be considered as a form of *infective heredity*, in the sense that the essential contribution of the infecting virus is to introduce into the infected host cell a functional material, which may be only a small portion of the infectious particle, and which contains the exact specifications for the unusual syntheses that will ensue. That is, the

viral material is not simply an activator of latent potentialities of the recipient cell, but a detailed blueprint, which in the cell takes its place within the hierarchy of cellular determinants of specificity, and whose genetic functions may sometimes be compatible, sometimes incompatible with normal cell functions.

According to this view, the infectious virus particles produced by a virus-infected or virus-carrying cell are simply one product of the pattern of synthesis determined in the virus-containing cell by the genetic apparatus, which includes both viral and host determinants, and which functions as an integrated whole. The significant acts of viral multiplication involve the replication of the genetic blueprints introduced into the cell by the virus. This replication may be integrated to a greater or lesser extent with the replication of the whole genetic apparatus of the cell. Instances range all the way from almost complete integration and synchronization, as with the prophage of temperate phages in lysogenic bacteria (Lwoff, 1953), to complete incompatibility, as with the most intemperate, destructive phages and animal viruses.

The mature, infectious particles appear to be the ultimate product of virus multiplication. Some of the replicating viral elements, together with nongenetic but specific materials produced under viral control in virus-infected cells, become incorporated into mature, nonmultiplying forms—the virus particles. These are recognized by their characteristic infectivity and organization. This assembly of virus particles removes some of the viral elements from the multiplying process and makes them suitable for introduction into new cells. It is analogous to spermatogenesis, which by a complex cytomorphogenetic process transforms a haploid cell into a form suitable for introduction of its nucleus into the egg cell. We consider this "dual hypothesis," which distinguishes two complementary and mutually exclusive processes, *replication* and *maturation*, as central to our understanding of virus biology.

Virus maturation will be a selectively advantageous process if it makes it easier for the virus to invade other hosts from without. The replicating form of a virus often appears to be noninfectious. By this we mean that, when extracted in this form, it is ineffective in initiating infection under conditions where the mature virus particle can do so. Yet, the lack of infectivity of the replicating virus may be only apparent. Under conditions that ensure protection from destructive agents and facilitate introduction into susceptible cells, we may succeed in observing initiation of infection by more or less incomplete virus particles, by their genetic components alone, or by multiplying viral elements extracted directly from cells prior to maturation. Instances of this sort will be discussed in the following sections. We shall return later to the relation between maturation and infectivity and to its significance for the general problem of infective heredity.

II. Multiplication of Bacteriophage

A. The Nature of the Replicating Phage Material

A tadpole-shaped phage particle attaches itself by the tip of its tail to the bacterial cell wall (Anderson, 1951). After a complex series of mutual interactions between phage and cell envelopes (Kellenberger and Arber, 1955; Kozloff *et al.*, 1957), the phage particle injects into the cell its DNA, together with some minor constituents (Hershey and Chase, 1952; Hershey, 1955, 1957). The protein shell is left at the surface and plays no further role in virus multiplication. This separation of the viral DNA from the protein shell, which is needed for attachment to cells, explains the "eclipse" of infectivity observed when extracts of newly infected bacteria are tested for ability to infect other cells (Doermann, 1952).

Following penetration of phage DNA, the infected cell may follow one of two paths,[1] depending on the genetic properties of the phage and on the environmental conditions: either the path of virus replication in "vegetative" form (Doermann, 1953), followed by virus maturation, cell lysis, and virus liberation; or the path of lysogeny (Lwoff, 1953), in which the cell multiplies, the virus persists in a noninfectious form and, as "prophage," becomes closely and persistently associated with the genetic apparatus of the bacterial cell (Jacob and Wollman, 1957). In the progeny of the lysogenic cells the prophage manifests itself occasionally by shifting to the vegetative form, which multiplies and produces mature virus and cellular lysis.

There is direct biochemical evidence that the phage material, both in its vegetative and in its prophage form, consists of DNA. The evidence concerning the vegetative form of phage derives mostly from work on coliphage T2. Isotope experiments have shown that in the cells that are going to produce phage there accumulates a pool of specific phage-precursor DNA (Hershey, 1956a,b), which is identifiable as phage DNA by its content of the unique pyrimidine (hydroxymethyl) cytosine, instead of cytosine (Wyatt and Cohen, 1952). In the pool, the phage-precursor DNA is not associated with any phage-precursor protein related to the proteins of the phage coat (Hershey and Melechen, 1957). Upon maturation, the phage-precursor DNA is removed at random from the pool and then becomes associated with phage-specific proteins. Synthesis of some protein (Cohen and Fowler, 1947; Burton, 1955; Tomizawa and Sunakawa, 1956) and, possibly, also of some specific RNA (Volkin and Astrachan, 1957) is required for the synthesis of phage DNA. These specific RNA and protein may be necessary intermediates in the replication of DNA. There may actually be a transfer of information from DNA to

[1] Other alternatives, such as abortive infection, or persistence of a nonmultiplying phage element in the cell, will not be considered here, insofar as they do not lead to multiplication.

non-DNA molecules, which will then carry, specified in their own chemical language, the whole specificity of the phage heredity (Delbrück and Stent, 1957). If so, some such non-DNA intermediate may be able to take over the control of DNA synthesis when the DNA itself is incapacitated, for example, by radioactive decay of P^{32} atoms in its nucleotides (Stent, 1955).

The evidence concerning the DNA nature of prophage comes from isotope experiments using coliphage λ. For this and other phages it has been possible to determine by bacterial crosses and by transduction (Lederberg and Lederberg, 1953; Jacob and Wollman, 1957) the presence and location of the corresponding prophages within the linear sequence of genetic determinants of the bacterial cell chromosome. The λ prophage can be inactivated in the lysogenic cell by the radioactive disintegration of P^{32} atoms incorporated into the cell. The rate of this inactivation is the same as the rate of inactivation of the infectivity of similarly labeled mature phage λ (Stent *et al.*, 1957). This provides a remarkable proof of the similarity of the content of essential DNA in the mature phage and in the prophage.

B. Infectious DNA from Phage Particles

These studies make it possible to identify the genetic material of the phage in its various states—mature, vegetative, and prophage—with a specific piece of DNA, which, at least in the form introduced into the cell by the mature particle, is probably not associated with genetically significant protein. Direct evidence has been obtained with phage T2 about the existence and size of this "master piece" of DNA and about its behavior and conservation in the process of replication (Levinthal and Thomas, 1957a,b; Hershey and Burgi, 1956).

Assuming that the nongenetic components of the mature virus particle are a protective and injecting device for the essential phage DNA, it can reasonably be expected that the DNA portion, extracted either from mature particles or from infected bacteria, may be able to initiate infection, if a system is available that permits penetration of the DNA into susceptible cells. At least for DNA from mature phage, the expectation seems to have been realized by the use of "protoplasts", that is, of cells deprived of part of their cell wall (Spizizen, 1957; Fraser and Mahler, 1957). According to these reports (which may not have excluded all possible pitfalls) the naked protoplast can be infected by disrupted phage, albeit with very low efficiency. We may recall in this connection that transformation phenomena with bacteria have established that fragments of bacterial DNA may be transmitted even to intact cells (Avery *et al.*, 1944; Hotchkiss, 1956). We may also mention here the phenomenon of zygotic induction (Jacob and Wollman, 1956), in which vegetative phage multiplication is initiated by the

penetration of some prophages into a susceptible protoplasm upon mating of a lysogenic bacterium with a nonlysogenic partner. It seems safe to assume that infection of a sensitive cell can be initiated by entry of the phage DNA in any one of its possible states. It is almost superfluous to point out that the possibility of infection of bacterial protoplasts with phage DNA promises new insight into the relation between structure and function of viral nucleic acid. Some protein component of the phage appears to play an essential role in the infection of protoplasts (Spizizen, 1957).

C. Kinetics of Replication of Vegetative Phage

If phage specificity throughout its reproductive cycles is embodied in DNA elements, the question arises of the kinetics of DNA replication in the course of vegetative multiplication of virus. By what mechanism does multiplication take place? Does it consist of repeated copyings of a single template, used over and over? Or does it involve a series of reduplications, in which the newly produced individuals serve in turn as sources for replication? In other words, is multiplication linear or geometric? The second alternative is verified by genetic observations on spontaneous phage mutations (Luria, 1951). These mutations occur only during multiplication; the resulting mutant phage particles are found among normal particles in the phage yield from single bacteria. The clonal distribution of the mutants in individual cells fits a distribution predicted by the hypothesis of geometric multiplication and incompatible with the hypothesis of a linear kinetics.

Current ideas on the structure and replication of DNA are compatible with its role as a geometrically replicated genetic material (Watson and Crick, 1953). A DNA molecule consists of two complementary polynucleotide chains. Its replication must involve the formation of two new complementary chains. The four chains will then yield two indistinguishable DNA molecules, presumably equal to each other in reproductive capacity.

Phage replication must also allow an exact homologous pairing between viral elements in order to account for the observed phenomena of genetic recombination. Pairing and recombination can also be accounted for in terms of mating during DNA replication (Delbrück and Stent, 1957; Levinthal and Thomas, 1957a), although more complex schemes invoking mating between non-DNA intermediates may ultimately prove preferable (Stent, 1958). The possibility of interactions similar to recombination between genetic elements of the phage and of the host is also suggested by a number of genetic observations, as discussed in Vol. II, chaps. VII and VIII of this work.

D. Functions of the Phage Genome

Viewed as functional DNA, the vegetative form and the prophage form of a bacterial virus are basically similar to fragments or portions of cellular

genetic material. Two consequences follow: First, like all genes, the phage DNA may be expected to control other cellular functions besides its own replication; second, the formation of mature phage may be considered as an expression of the specific genetic function of the phage DNA. Both predictions are supported by available evidence.

1. *Conversions of Cellular Properties by Phage*

A number of cell properties that are not obviously related to virus production are controlled by phage genes. Most remarkable among these is the control of the composition of the cell wall, which manifests itself by specific changes in cellular antigens upon phage infection. This phenomenon has been studied mostly in the genus *Salmonella* (Iseki and Sakai, 1953; Uetake *et al.*, 1955). In what is probably a typical instance, infection with a certain phage results in the appearance, within a few minutes, of somatic antigen 15 and in the equally prompt suppression of the production of antigen 10. This change occurs both in cells in which the phage multiplies vegetatively leading to cell lysis and in cells that survive infection and in which the phage becomes prophage (Uetake *et al.*, 1958). It occurs even in infection with a virulent phage mutant that lyses every infected cell. The reverse change, from antigen 15 to antigen 10, follows the loss or removal of the phage from the carrier cells.

Clearly, the relation between the phage DNA and the specific constituents of the somatic antigens is no more and no less obvious than the relation between the DNA of a transforming principle and the capsular polysaccharides in *Pneumococcus* (Avery *et al.*, 1944), or, for that matter, than the relation between any gene and the ultimate product of its activity in any cell.

There is a whole series of these "conversions" of cell properties by phages, ranging from the production of diphtheria toxin (Freeman, 1951) to the ability to support multiplication of other phages (S. Lederberg, 1957). It was believed at first that such new properties required the presence of an established prophage; hence the name of "lysogenic conversions" (J. Lederberg, 1955). We realize now, however, that these conversions of cell properties are expressions of heterocatalytic activities that may be exerted by all functional states of phage within a cell.

2. *Biosyntheses Related to Phage Replication*

It seems reasonable to attribute to the heterocatalytic functions of phage also the appearance in phage-infected cells of new enzyme activities related to the needs for synthesis of phage DNA. The most remarkable instance is the appearance of an enzyme that catalyzes the hydroxymethylation of deoxycytidylic acid (Flaks and Cohen, 1957) in bacteria infected with the

coliphages of the T2 group, which contain the hydroxymethylated nucleotide (Wyatt and Cohen, 1952). The enzyme is clearly required for synthesis of phage DNA itself. Although the enzyme may conceivably be present in inactive form in the bacteria and be activated by phage infection, as in the case of a bacterial deoxyribonuclease (Pardee and Williams, 1952; Kozloff, 1953), it seems more probable that the enzyme is synthesized anew under the genetic control of the incoming phage DNA. Similar mechanisms may underlie the restoration or expansion of thymine synthesis in an almost thymineless bacterial strain following infection with phage (Barner and Cohen, 1955).

3. *Syntheses Related to Phage Maturation*

If we accept the concept of phage DNA acting as genetic material in integration with the cell genome and controlling heterocatalytically a number of biosynthetic processes, it becomes natural to consider also the proteins of the mature phage particles as special products of the functional activity of the phage genome.

The proteins of a phage particle comprise a variety of antigenically distinct fractions (Lanni and Lanni, 1953), some of which are probably active enzymatically (Brown and Kozloff, 1957). Some are located in the head of the phage, others, in the tail. The tail proteins include the organ of phage attachment to the cell. When a bacterium is infected with two related phages, whose tail proteins differ in antigenic specificity or in requirements for adsorption cofactors (Anderson, 1945), the progeny particles exhibit "phenotypic mixing" (Novick and Szilard, 1951). That is, the specificity of the tail proteins may correspond, not to the genetic characteristics of the phage particle that carries them, but to the characteristics of the other phage type that was growing in the same cell, or to a mixture of the two. The association between genetic and phenotypic properties is almost random (Streisinger, 1956; Brenner, 1957). This indicates that the two kinds of tail protein are synthesized "at large" in the infected cells and are then utilized, as available, in assembling the coats of the maturing phage, in the same way as gene products controlled by different allelic genes may be utilized side by side in a heterozygous cell, or as gene products controlled by genetically different nuclei in a heterocaryotic cell or mycelium.

Other specific proteins, besides those destined to become part of the mature particles, are produced in the process of phage maturation. These include a number of agents that act enzymatically to dissolve the surface layers of bacteria, some digesting capsular polysaccharides (Adams and Park, 1957), others attacking the bacterial cell wall (Huppert and Panijel, 1957; Murphy, 1958; Jacob and Fuerst, 1958). Such enzymes play a role in bacterial lysis, in the release of the newly formed phage, and in

the removal of external cell constituents that might interfere with attack on new host cells.

E. Phage Maturation and Infective Heredity

The interpretation of phage maturation as the terminal assembly of a specific core of viral DNA with specific proteins synthesized by the virus-infected cell under viral control leads to several predictions as to possible events that may take place at maturation or affect the occurrence of maturation.

1. *Transduction*

In the course of vegetative replication and maturation, a phage particle may occasionally come to include in its protein shell some fragment of the bacterial genome. This gives rise to "transduction," as observed in *Salmonella* (Zinder, 1953), in the coli-dysentery group (Lennox, 1955), and probably also in the genus *Bacillus* (Brown *et al.*, 1955). In this type of transduction, the phage can transfer from one cell to another any group of closely linked host genes. If the cell survives infection, it may show one or more of the transduced characters.

In at least one other instance, with phage λ, the only host genes that can be transferred are some that were chromosomal neighbors of the prophage in a lysogenic cell, including a group of factors controlling utilization of galactose (Morse *et al.*, 1956). Here the transducing particles appear to have incorporated the fragment of host genome in the place of a portion of the phage genome itself (Arber *et al.*, 1957). This transducing phage thereby becomes incomplete and ineffective in initiating its own reproduction, although it can still produce cell lysis. This "defective" phage has become a specific transducer of the galactose determinants, which behave here as infective genetic factors. There is now evidence (Luria *et al.*, 1958) that other instances of transduction may also reflect associations of bacterial genes with defective phage.

2. *Defective Prophages*

If maturation is the culmination of a process of specific phage-controlled biosynthesis, we may expect that both environmental agents and genetic changes will affect the very occurrence of maturation. An example of an environmental effect is the specific prevention of successful phage assembly and maturation by inhibitors such as the acridine dye, proflavine (DeMars, 1956). The defective prophages, on the other hand, provide examples of genetic effects on maturation (Appleyard, 1954; Jacob and Wollman, 1956a).

Here, lysogenic bacteria lose by mutation the ability to produce normal mature phage, without losing some of the prophage-controlled properties, such as immunity to lysis by superinfection or production of phage-controlled antigens. The mutations to defectiveness occur in the prophage itself, and the nondefective prophage form may be restored by back mutation. With some phages the defect leads to incomplete maturation. Lysis will then result either in production of no recognizable phage elements, or of fragments of phage coats, or of some particles that carry the genetic defect (Appleyard, 1956).

The notable fact is that the defective prophages, being genetically competent in other respects, but incompetent to determine production of infectious virus, have lost their "viral" aspect. They have become operationally indistinguishable from any other fragments of genetic material of the cell. Yet, we know the exogenous origin and the potential transmissibility of these genetic determinants, revealed in some cases by their back mutations to nondefectiveness. Since we have independent evidence, from transduction, that most or all elements of the bacterial genome are transferable from cell to cell if a suitable viral vehicle is available, we are led to ask how many of these genetic elements either possess or can acquire by mutation the potentiality to determine their own specific incorporation into a viral vehicle formed under their own control. That is, we ask whether all portions of a cell genome might become viruses and whether in so doing they would manifest an ever present potentiality, or acquire a novel cytomorphogenetic function, or recover a function that had been lost by mutation.

There are in bacterial genetics a number of situations that can be interpreted in terms of special genetic elements or "episomes" (Jacob and Wollman, 1958), with the ability to behave at times as chromasomal elements, at other times as units multiplying vegetatively in the bacterial cell. Prophages may be considered as a category of such episomes capable of assuming an effectively transferable form. Other episomes might conceivably acquire this capability by mutation.

As we interpret phage infection as genetic parasitism, we identify phages more and more closely with wandering portions of the cell genome. More generally, we must ask what role infective heredity has played and may still be playing in the evolution of genetic systems (J. Lederberg, 1952; Luria, 1953).

III. Multiplication of Tobacco Mosaic Virus

A. RNA as the Initiator of Infection

Little is known about the multiplication of tobacco mosaic virus (TMV) at the cellular level, but several lines of evidence are relevant to our discussion.

As far as the initiating material in infection is concerned, there is clear evidence that this is the RNA portion, which in the mature virus particles is contained within a spirally assembled shell of protein units (R. E. Franklin *et al.*, 1957). The purified RNA fraction extracted from the virus particles can initiate infection by itself, although less efficiently (per unit weight of RNA) than the complete nucleoprotein particles (Gierer and Schramm, 1956; Fraenkel-Conrat, 1956).

The early development of the infection, as revealed by changes in the radiation sensitivity of the virus-producing capacity of the infected cells, shows significant differences between infection with complete virus and infection with the RNA fraction alone (Siegel *et al.*, 1957). In infection with complete virus, there is an early phase during which the sensitivity to ultraviolet light is very high. In infection with the viral RNA alone, this early phase is missing; the whole situation evolves as though the process started directly at a later stage. These observations suggest that in infection with complete virus particles a first necessary step is the release of RNA from its protein shell, so that it can act as the *primum movens* in the process of virus multiplication.

It seems probable that with TMV infection, as with phage infection, the multiplying form of the virus consists of nucleic acid not associated with the protein found in the mature product. Here again, the viral protein may be a specific product of the virus-infected cell, utilized for coating the essential nucleic acid and providing it with a protective apparatus that enhances its chances of successful transmission to other plants. It is conceivable that transmission of virus from cell to cell within an infected plant may occur by the transfer of RNA elements, rather than of complete nucleoprotein particles.

B. TMV Protein and Virus Maturation

What is known of the properties and biosynthesis of TMV protein fits the hypothesis that we have outlined. In infected cells, TMV protein is found, not only in the virus particles, but also as a noninfectious material, presumably not associated with viral RNA or at least readily separated from it by extraction (Jeener, 1956). Isotopic experiments show that at least some of this noninfectious viral protein behaves as a true precursor of the virus particles, into which it becomes incorporated (Van Rysselberge and Jeener, 1957).

The viral protein is made up of small subunits, about 17,000 in molecular weight. These appear to be uniform in structure and composition, at least within the limits of present analytical methods (Knight, 1957). The protein extracted from infected cells or from virus particles has a remarkable tendency to aggregate, under suitable conditions, either alone or around a core of nucleic acid, to give the typical helical arrangement of the protein in the virus particle (Schramm, 1947).

Complete virus particles can be reconstituted by recombining RNA and protein separately extracted from virus. The reconstituted particles have some infectivity (Fraenkel-Conrat and Williams, 1955). If RNA from one virus strain and protein from a different strain are combined, the progeny to which they give rise has the genetic characteristics contributed by the RNA.

Thus, the TMV protein appears to be a specific material, without intrinsic genetic function, produced under the genetic control of the viral RNA, and utilized in the morphogenetic process of virus maturation.

Here again, this time with a typical RNA virus, the nucleic acid must be considered as the primary genetic material of the virus, and the mature particle as one product of the genetic activity of the virus. The occurrence of other virus-related proteins, which are probably not precursor proteins, indicates that the mature virus is not the only specific product of virus-infected cells. Which other cell functions this virus may control is not known.

The amount of RNA in a TMV particle can probably carry more genetic information than is needed to determine the specificity of the viral protein. The additional information, if any, may control other functions of the virus in the cell. We may also find, in such an RNA virus, some "transduced" elements of host cell RNA.

C. Other RNA Viruses

A few scattered observations on other RNA-containing viruses support the conclusions reached for TMV virus. With poliovirus, Mengo, and West Nile encephalitis viruses, successful transmission of infection has been reported by means of an RNA fraction extracted from infected cells (Colter *et al.*, 1957a,b). Conversely, with turnip yellow mosaic virus, there is found in infected cells a fraction of particles, similar to the infectious virus particles in size, structure, and protein composition, but without RNA and completely noninfectious (Markham and Smith, 1949). These particles are probably a product of faulty maturation, the essential RNA failing to be enclosed into the protein shell. In the complete virus particles, as well as in the noninfectious ones, the protein actually appears to constitute a shell composed of repeated subunits (Klug *et al.*, 1957). It seems a useful hypothesis to assume that with these viruses, and probably also with others like poliovirus, whose particles contain only RNA and protein, the synthesis of virus protein is always a terminal event, leading to the maturation of the virus and to the cessation of the reproduction of its essential genetic material.

IV. Multiplication of Animal Viruses

A. Myxovirus Group

The myxovirus group includes the viruses of influenza, Newcastle disease, mumps, and fowl plague. The particles of these viruses contain at least three antigenically distinct fractions : an RNA-protein element, called the S (or G) antigen; a hemagglutinin (HA) element; and a lipid-containing fraction. Extraction with ethyl ether destroys the particles and permits separation of the S and HA fractions from other materials (Hoyle, 1952), some of which cross-react serologically with antigens of the host tissue (Knight, 1946).

The changes undergone by the virus particles upon initiation of infection are not yet definitely established (Hoyle, 1957; R. M. Franklin *et al.*, 1957). It is known, however, that the various components appear at different times and in different parts of the cell, the S antigen, first, in or around the nucleus, the HA in the cytoplasm (Liu, 1955; Breitenfeld and Schäfer, 1957). Infectious virus particles appear later than the S and HA elements. Complete particles are never seen within the cells, but only at the cell surface (Morgan *et al.*, 1956). All new infectious virus present at any one time in the infected cells is subject to inactivation by external agents, such as antibody (Rubin *et al.*, 1957), and can be released readily from the cell by treatment with a receptor-destroying enzyme. The unescapable conclusion seems to be that the various virus constituents are formed at different sites within the cell and that their assembly and maturation take place as terminal processes at the cell surface. The assembly process, however specific it may be, permits or even requires the incorporation into the virus particles of certain materials whose antigenic specificity is host-determined. Such a relatively unspecific process of assembly may at least partly be responsible for the genetic complexity of virus particles produced in cells that receive a mixed infection with two related viruses of this group (Burnet, 1955). It also provides opportunities for transduction like phenomena in these viruses.

The RNA-containing S element seems the natural candidate for the primary genetic function in these viruses. Isotopic studies on influenza and other myvxoviruses suggest a breakdown of the infecting particles at the surface of the infected cell and an initiation of growth by multiplication of S antigen (Hoyle, 1957). This evidence is somewhat beset by technical difficulties, due to the relative instability of the influenza virus. Whether the RNA component can initiate infection by itself, and whether it becomes separated from viral protein as part of the initiation of infection, remain subjects for future study.

B. Other Viruses

The rather fragmentary observations on the multiplication of animal viruses of other groups, although they add little to the picture developed in the preceding pages, are fully compatible with it. There is, in the first place, a general finding of an eclipse of infectivity following infection. This may be taken as an indication of a drastic change in the structure of the virus in passing to the multiplying state. There is also ample cytochemical and microscopic evidence for a series of stages, different for different groups of viruses, through which the virus materials must go before becoming organized into mature virus particles. Often, the first virus materials to appear in an infected cell are seen in electron micrographs as an undifferentiated matrix, within which the typical virus particles are then formed by a stagewise process of maturation (Gaylord and Melnick, 1953). The frequent intranuclear or perinuclear location of these foci of virus production suggests that some interaction with the host cell DNA may be required to initiate reproduction, even for viruses of the RNA group. It seems possible that production of an RNA-containing virus may require some genetic alteration involving a change in the cellular DNA.

With viruses of the psittacosis group, microscopic observations have suggested that multiplication entails a binary fission of viral elements, which differ morphologically from the mature virus particles (Sigel *et al.*, 1951). Such a finding, if correct, would by no means be incompatible with the hypothesis of a multiplying form of the virus distinct from the infectious mature particle. The elementary act of virus multiplication must always be a reduplication of the genetic elements of the virus. It is not surprising that the reduplication process of vegetative (and possibly noninfectious) virus elements may express itself in morphologically recognizable acts of binary fission. Repeated reduplications of virus elements must underlie the exponential kinetics of virus production observed in some viral infections of individual cells (Dulbecco and Vogt, 1953).

For some insect viruses, a complex reproductive process has been postulated on the basis of morphological studies (Bergold, 1953). Although the basic mechanisms are still doubtful, the existence of separate phases of replication and maturation seems very probable.

V. Virus Multiplication, Cell Function, and Cell Organization

A. Restatement of the Dual Hypothesis

The picture of virus multiplication outlined in the preceding sections has a number of unifying features. In all cases, multiplication appears to be initiated by a genetic portion of the virus particles, which contains nucleic acid and which is either noninfectious or, at least, less infectious than the mature virus by the available tests. The production of new virus entails both

the replication of the genetic material, a clearly antocatalytic process, and the maturation of new virus, in which the genetic elements stop replicating and become assembled into virus particles, by joining up with materials synthesized under the heterocatalytic control of the virus. Cell-specific materials, either genetically competent (as in transduction) or presumably with structural functions (as in influenza viruses), may also become included into virus particles. In its functional state within a cell, the genetic material of a virus can control, not only the synthesis and assembly of constituents of the mature virus particles, but also the production of essential enzymatic mechanisms and other biochemical processes, which manifest themselves as altered cell functions. Some of these functions of a virus may be instances of pleiotropic gene action, by which genetic functions essential for virus multiplication accidentally affect other cell functions. It is equally conceivable, however, that a virus, as a transmissible fragment of cell heredity, may contain the genetic determinants of functions unrelated to its own perpetuation as virus.

A number of questions may now be raised: How does virus multiplication lead to the cellular dysfunctions observed in many viral infections? Which cellular properties are determined by genetic elements that can act as viruses? And what relationship exists between these elements and the other genetic elements of the cell?

B. Cell Damage and Virus Multiplication

Cellular dysfunction may result from any of the phases of interaction between viruses and cells. With certain "intemperate" phages, for example, the mere attachment of a virus particle, even unable to multiply, can cause irreparable damage and cell death. Other changes in cell properties, like the antigenic changes in phage-infected bacteria, are observed whenever viral multiplication occurs, either in the vegetative or the prophage state. Still other forms of damage, such as lysis of bacteria by phage or destruction of animal cells by certain viruses, are probably tied up with the process of virus maturation.

Replication of a virus in a noninfectious form, either as vegetative virus or as provirus, is often compatible with continued cell life and cell division, as in lysogenic bacteria. Virus maturation, which involves extensive changes in the pattern of cellular biosynthesis, is probably more directly related to cellular damage leading to cell death. Even in some proliferative virus diseases the mature, infectious virus particles might be produced only in a few cells that are prevented from further growth. At least for Rous sarcoma, however, there is now some evidence of production of mature virus by living, multiplying cells (Rubin and Temin, 1959).

In general, cellular damage appears to be an incidental manifestation of virus infection, even though it is a frequent correlate of virus maturation. Cellular dysfunctions of a variety of types, ranging from simple metabolic alterations in some lysogenic bacteria to unrestricted cell proliferation in virus-induced tumors, must be considered as the expression of the genetic functions of the virus acting in integration with the host cell genome.

These virus-controlled functions are not necessarily different from cellular functions that may arise, be modified, or be suppressed by genetic changes such as mutations. Once we visualize virus infection as a form of infective heredity, the problem of the possible determination of apparently normal functions by virus-like elements reduces itself to the problem of the potential ability of various genetic elements of the cell to behave as viruses: that is, to control their own maturation into readily transferable forms. At present, this problem can be defined precisely only for phages, which, by their reduction to chromosome-linked prophages, their interactions with neighbouring chromosomal elements, and their mutations to defectiveness, demonstrate the possible transitions between virus and chromosomal element. In bacteria, at least, transformation and transduction give proof of the intrinsic transferability of all the genetic material as functional DNA. With other cells, the occurrence of latent infections and the activation of unsuspected viruses upon transfer of tissue extracts into new hosts have repeatedly suggested the possibility of a transformation of cell components into viruses.

Specific cellular components released by growing cells may play a role in growth regulation phenomena (Weiss, 1955), as well as in tissue compatibility (Billingham *et al.*, 1956). It is conceivable that some of these regulatory substances may contain nucleic acids and may be able to reproduce in cells into which they gain access.

C. Viruses and Cellular Constituents

Our discussion has led us to consider the natural relationship of viruses to constituents of normal cells. We are not stretching our imagination too far if we consider the phage DNA as a transmissible fragment of bacterial DNA. Some of the RNA viruses, on the other hand, may ultimately prove related or homologous to cell microsomes, which, as part of the cytoplasmic reticulum, are probably the carriers of the coded determinants for protein synthesis in the cell protoplasm (Simkin and Work, 1957).

A detailed discussion of virus origin would be outside the scope of this chapter. We may point out, however, that even if viruses are genetically related to certain cell constituents, it is unjustified to expect a detailed homology between the genetic structure and physiological functions of a virus and the structure and functions of constituents of the cells in which

we happen to observe it. A great deal of independent, divergent evolution may have taken place both in virus and in cells since a virus first arose from a cell constituent through the acquisition of an apparatus for successful transfer from cell to cell. Conceivably, most cells may have great latitude in the variety of genetic elements, exogenous or endogenous, whose reproduction they are potentially capable of supporting. Only those elements that have evolved both a mechanism for successful transfer and a set of functions observable in other cells may be recognizable as viruses. Transferability of a virus may be extended by mutation to cells that are phylogenetically very distant from one another and from the (hypothetical) cell whence the virus first came. Suffice it to recall the plant pathogenic viruses that can multiply in the cells of plants and of insects (Maramorosch, 1955).

To return to virus multiplication, the hypothesis of a genetic relationship between viruses and cellular organelles does not in itself contribute to our present understanding of virus multiplication. In fact, we know very little as yet about the mechanism of reproduction of these organelles. Rather, we may be certain that the study of virus multiplication will, directly and indirectly, be a major contributor to the elucidation of the mechanisms of replication of the basic units of life.

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